Dispatches

Collective Migration: Spatial Tension Relief

Collective epithelial cell migration facilitates formation and maintenance of continuous sheets that line the surfaces and cavities of glands and tissues. By screening Rho GTPase regulators, myosin-IXA RhoGAP was identified as a key requirement for cell–cell adhesions that permit collective migration.

Jenifer M. Wood and Michael F. Olson*

The division between a tissue and its immediate surroundings is typically created by uninterrupted sheets of epithelial cells. An example of this is also the answer to a pub quiz question that everyone knows (Question: What is the human body's largest organ? Answer: The skin). As the primary functions of epithelial layers are to protect and ensure homeostasis in underlying tissues, it is essential that the integrity of the epithelial sheet is maintained. This poses a challenge during morphogenesis as epithelial cells would need to remain coherent to help maintain a barrier whilst migrating, a process often thought to require loss of cell–cell contacts. This apparent dilemma is overcome, however, by a phenomenon known as collective cell migration, where cells retain many properties of singly migrating cells but remain cohesive and move together [1]. How cells migrate individually has been extensively studied, but the mechanisms involved in collective cell migration are less well understood. In this issue of *Current Biology*, Omelchenko and Hall [2] extend our understanding of this process by identifying the RhoGAP activity of myosin-IXA as a key regulator of cell–cell contacts required for collective cell migration.

Although relatively little is known about the molecular regulators of collective cell migration, it is a vital process in development, tissue repair and cancer progression. Early stages in development across many species depend on the movement of groups of cells; for example, the migration of border cells in *Drosophila* ovaries [3] and lateral line primordium cells in zebrafish [4], as well as gastrulation in multicellular organisms [5], to name a few. In wound healing, collective cell migration is required for epithelial cells to move as a contiguous unit to close the wound [6]. Invasion of cancer cells into tissue surrounding a primary tumour is a key step in tumour progression and it has been observed that this may occur individually as single cells or collectively as clusters or strands of cells [7,8]. Therefore, elucidating the mechanisms involved in regulating collective cell migration is essential to enable further advances to be made in understanding development and cancer progression.

The distinguishing feature of collective cell migration is that cells remain physically connected. The obligatory maintenance of cell–cell adhesions for collective cell movement is mediated primarily by adherens junctions composed of transmembrane cadherin proteins, in which extracellular domains bind cadherins on adjacent cells and intracellular domains interact with adaptor proteins that link adherens junctions to the actomyosin cytoskeleton [9,10]. Although these junctions are robust they are also highly dynamic and plastic, allowing cells to move in relation to one another whilst remaining strongly associated. It has been suggested that these junctions not only ensure a physical connection between cells during migration, but also permit the transmission of mechanical forces through groups of cells resulting in coordinated movement initiated by cells at the front of the group [11].

The actomyosin cytoskeleton is important for the formation and stability of cell–cell junctions. As actin polymerises at the leading edges of cells, the membrane protrusions generated are often the initial points of cell–cell contact where adherens junctions first form [12]. Actomyosin filaments also assist in cadherin protein clustering, allowing stronger points of contact to develop [13]. After initial contact, further extensions of the actin-rich protrusions into adjacent cells increase the contact area, thereby elevating the potential number of adherens junctions that can form [12] and consequently strengthening connections between adjacent cells. Therefore, actomyosin cytoskeletal dynamics are critically important for the initial formation, stability and rearrangement of these adherens junctions that enable collective migration.

Although it is clear that dynamic changes in the actomyosin cytoskeleton play central roles in many aspects of cell–cell adhesion, what is responsible for regulating these changes? The Rho family of GTPases, including RhoA, Rac1 and Cdc42, are well established as key regulators of the actin cytoskeleton and act as molecular switches by cycling between their GTP- and GDP-bound states [14]. Once bound to GTP they interact with and regulate a number of effector proteins, many of which are involved in actin dynamics [15]. Due to the major involvement of Rho GTPases in regulating the actomyosin cytoskeleton, it would be anticipated that they would also have roles at cell–cell junctions. Indeed, members of the family have been found to localise to cell–cell adhesions, and their activity is required for these adhesions [16]. Additionally, the direct interaction between cadherin molecules at cell–cell junctions leads to the activation of Rac1 [17].

If we were to take this pathway a step further back, the question would be what regulates the Rho GTPases at cell adhesion sites? Three main classes of proteins control the bound nucleotide state and therefore the activity of Rho proteins: guanine nucleotide exchange factors (GEFs), which activate Rho proteins by catalysing the exchange of GDP for GTP; GDP dissociation inhibitors (GDIs), which prevent the exchange of GDP for GTP and the consequent Rho protein activation; and GTPase-activating proteins (GAPs), which inactivate Rho proteins by stimulating the hydrolysis of GTP to GDP. The localisation and activity of these regulators enables the

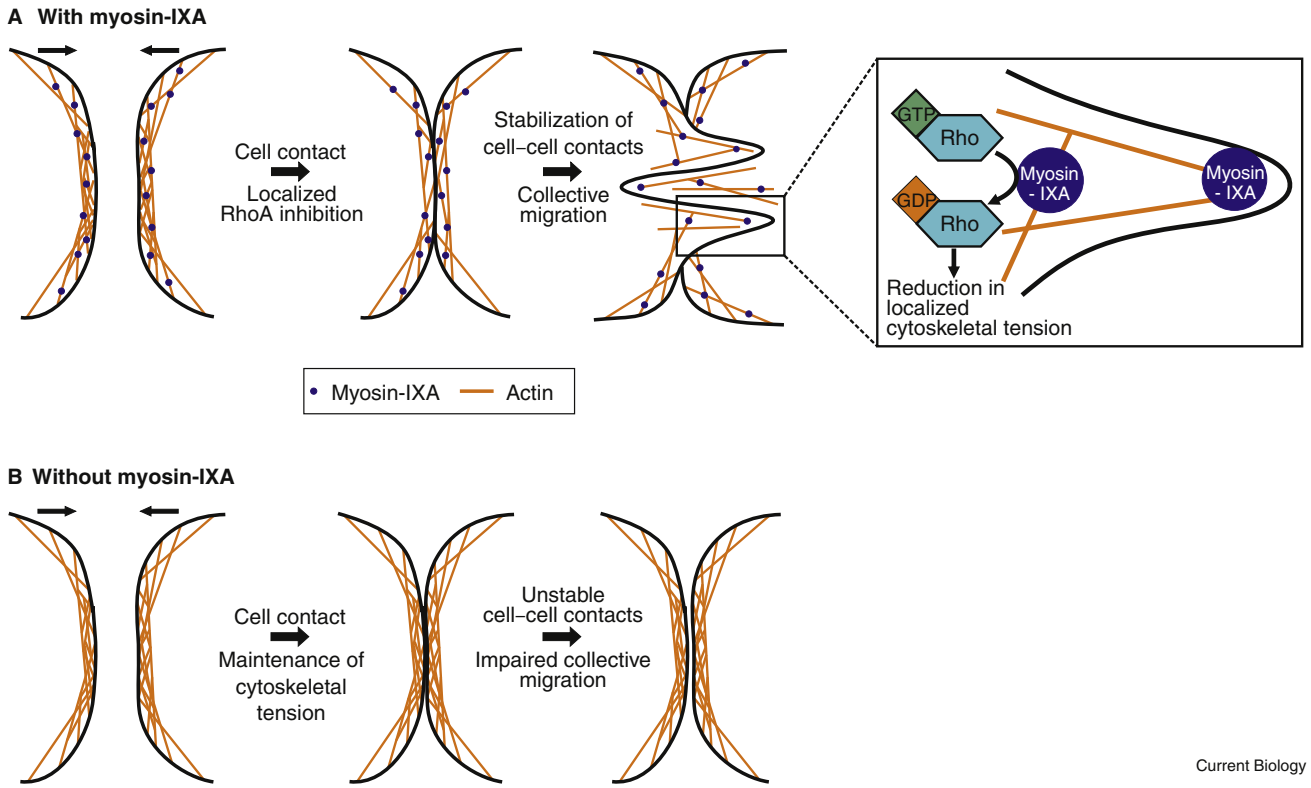

Figure 1. Myosin-IXA induces localised RhoA inhibition at points of cell–cell contact reducing cytoskeletal tension and stabilising cell–cell contacts essential for collective cell migration.

(A) Myosin-IXA is recruited to sites of cell–cell contact via its motor domain where it locally inhibits RhoA through its RhoGAP activity, leading to a reduction in cytoskeletal tension, formation of radial filaments and stabilisation of cell–cell contacts, thereby enabling collective cell migration. (B) In the absence of myosin-IXA, cytoskeletal tension is maintained, resulting in unstable cell–cell contacts and impaired collective migration.

magnitude and duration of Rho GTPase signalling to be tightly and spatially controlled [15]. Although numerous GEFs, GDIs and GAPs have been described, the understanding of their possible involvement in cell–cell adhesion and collective cell migration is in its infancy. The study by Omelchenko and Hall [2] aimed to fill this knowledge gap by undertaking a screen to identify regulators of Rho-family GTPases that are necessary for the collective migration of epithelial cells, and successfully identified the RhoGAP and actin motor protein myosin-IXA as one such regulator.

Omelchenko and Hall [2] found that, in the absence of myosin-IXA, cells were unable to maintain cell–cell adhesions and migrate collectively (Figure 1). Interestingly, it was only cell–cell contacts in a dynamic state, such as those in collectively migrating epithelial cells, that were affected, whereas assembly of junctions in non-migrating cells was unaffected by myosin-IXA depletion. After further investigation of this phenomenon, they discovered that myosin-IXA is recruited to points of cell–cell contact through its actin-binding motor domain during early stages of junction formation. Here it is required for the reorganisation of actin into radial actin filaments to allow stabilisation of the newly formed cell–cell junctions, a function dependent on its RhoGAP domain. The myosin-IXA motor domain contributes to localisation of the protein to actin bundles, allowing for tight spatial regulation of RhoA activity. Consistent with this possibility, imaging analysis revealed elevated RhoA activity in myosin-IXA knockdown cells relative to control cells at nascent cell–cell contacts formed during cell collisions. They therefore conclude that myosin-IXA spatially limits RhoA activity and actomyosin contractile tension at developing cell–cell adhesions, which is essential for robust cell–cell adhesion and consequent collective migration of epithelial cells. The results reported here on the cellular role of myosin-IXA in regulating epithelial cell–cell adhesions are consistent with observations in a myosin-IXA knockout mouse where defects were seen in junction formation and cell morphology in the epithelial lining of cerebrospinal fluid-filled ventricles in the brain [18].

Omelchenko and Hall's findings [2] confirm the importance of tight spatial control of RhoA activity and actomyosin contraction at cell–cell junctions and the importance of establishing and maintaining cell adhesions in collective migration, further highlighting the vast number of proteins and complexity of signal networks that are essential for ensuring the integrity of an epithelial layer during morphogenesis. We are still a long way from a complete understanding of the molecular players involved in the formation and maintenance of cell–cell junctions and how they contribute to collective migration; however, this report is a significant step forward. Due to the large number of Rho GTPase regulators, it is certain that there will be

additional pieces in the collective cell migration puzzle. As collective epithelial cell migration is an integral part of development and cancer progression, further advances in the area will aid our understanding and could ultimately lead to improved treatment of developmental diseases and cancer.

References

1. Revenu, C., and Gilmour, D. (2009). EMT 2.0: shaping epithelia through collective migration. Curr. Opin. Genet. Dev. *19*, 338–342.
2. Omelchenko, T., and Hall, A. (2012). Myosin-IXA regulates collective epithelial cell migration by targeting RhoGAP activity to cell-cell junctions. Curr. Biol. *22*, 278–288.
3. Geisbrecht, E.R., and Montell, D.J. (2002). Myosin VI is required for E-cadherin-mediated border cell migration. Nat. Cell Biol. *4*, 616–620.
4. Ghysen, A., and Dambly-Chaudiere, C. (2004). Development of the zebrafish lateral line. Curr. Opin. Neurobiol. *14*, 67–73.
5. Keller, R. (2005). Cell migration during gastrulation. Curr. Opin. Cell Biol. *17*, 533–541.
6. Farooqui, R., and Fenteany, G. (2005). Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. J. Cell Sci. *118*, 51–63.
7. Alexander, S., Koehl, G.E., Hirschberg, M., Geissler, E.K., and Friedl, P. (2008). Dynamic imaging of cancer growth and invasion: a modified skin-fold chamber model. Histochem. Cell Biol. *130*, 1147–1154.
8. Olson, M.F., and Sahai, E. (2009). The actin cytoskeleton in cancer cell motility. Clin. Exp. Metastasis *26*, 273–287.
9. Vasioukhin, V., and Fuchs, E. (2001). Actin dynamics and cell-cell adhesion in epithelia. Curr. Opin. Cell Biol. *13*, 76–84.
10. Pokutta, S., Drees, F., Yamada, S., Nelson, W.J., and Weis, W.I. (2008). Biochemical and structural analysis of alpha-catenin in cell-cell contacts. Biochem. Soc. Trans. *36*, 141–147.
11. Ganz, A., Lambert, M., Saez, A., Silberzan, P., Buguin, A., Mege, R.M., and Ladoux, B. (2006). Traction forces exerted through N-cadherin contacts. Biol. Cell *98*, 721–730.
12. Gavard, J., Lambert, M., Grosheva, I., Marthiens, V., Irinopoulou, T., Riou, J.F., Bershadsky, A., and Mege, R.M. (2004). Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. J. Cell Sci. *117*, 257–270.
13. Adams, C.L., Chen, Y.T., Smith, S.J., and Nelson, W.J. (1998). Mechanisms of epithelial cell-cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin-green fluorescent protein. J. Cell Biol. *142*, 1105–1119.
14. Jaffe, A.B., and Hall, A. (2005). Rho GTPases: biochemistry and biology. Annu. Rev. Cell Dev. Biol. *21*, 247–269.
15. Spiering, D., and Hodgson, L. (2011). Dynamics of the Rho-family small GTPases in actin regulation and motility. Cell Adh. Migr. *5*, 170–180.
16. Nakagawa, M., Fukata, M., Yamaga, M., Itoh, N., and Kaibuchi, K. (2001). Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell-cell adhesion sites. J. Cell Sci. *114*, 1829–1838.
17. Nola, S., Daigaku, R., Smolarczyk, K., Carstens, M., Martin-Martin, B., Longmore, G., Bailly, M., and Braga, V.M. (2011). Ajuba is required for Rac activation and maintenance of E-cadherin adhesion. J. Cell Biol. *195*, 855–871.
18. Abouhamed, M., Grobe, K., San, I.V., Thelen, S., Honnert, U., Balda, M.S., Matter, K., and Bahler, M. (2009). Myosin IXa regulates epithelial differentiation and its deficiency results in hydrocephalus. Mol. Biol. Cell *20*, 5074–5085.

Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow G61 1BD, UK.
*E-mail: m.olson@beatson.gla.ac.uk

DOI: 10.1016/j.cub.2012.01.026

Brain Development: Neural Signature Predicts Autism's Emergence

A new study has found that neural sensitivity to eye gaze in early infancy is associated with subsequent development of autism. This discovery provides a much-needed biomarker for autism spectrum disorder prior to emergence of behavioral symptoms.

Kevin A. Pelphrey and James C. McPartland

Autism spectrum disorder (ASD) is a common, early-onset neurodevelopmental disorder characterized by difficulties in social interaction and communication and repetitive or restricted interests and behaviors [1]. ASD displays great phenotypic heterogeneity and etiological diversity, but since its original description, social dysfunction has been its hallmark and unifying feature [2]. This social dysfunction is revealed by abnormalities in both simple behaviors, such as sharing gaze, and more complex social behaviors, such as triadic attention sharing. Anomalies of social perception, unlike communication problems or repetitive behaviors that are present in numerous disorders (such as anxiety or expressive language impairment), are unique to ASD and are documented across sensory modalities. Autism is a developmental disorder; early deficits derail subsequent experiences, thereby canalizing development towards more severe dysfunction and creating sequelae in additional domains of function. Consequently, the lack of reliable predictors of the condition during the first year of life has been a major impediment to the effective treatment of ASD. Without early predictors and in the absence of a firm diagnosis until behavioral symptoms emerge, treatment is often delayed for two or more years.

In response to the urgent need for a sensitive and specific biomarker of ASD, many research groups from around the world have been intensely studying patterns of infant development. These studies have involved prospective longitudinal studies of infant siblings of children with ASD. Such designs use a comparison group of infant siblings without familial risks (the low-risk group) to gather longitudinal information about developmental trajectories across the first three years of life, followed by clinical diagnosis at 36 months. As recently reviewed by Rogers [3], the behavioral work to date, using measures such as eye-tracking and social probes, has failed to detect atypical social development in the first months of life, instead portraying "autism as a disorder involving symptoms across multiple domains with a gradual onset that changes both ongoing developmental rate and established behavioral patterns across the first 2–3 years of life".

The findings presented in this issue of *Current Biology* by Elsabbagh and colleagues [4] challenge this notion and remind us that our ability to study development is contingent upon the power of our methods of inquiry. Their provocative results suggest that investigation at the neural systems level may reveal distinctions inaccessible to behavioral assays alone. They tested the hypothesis that neural sensitivity to eye gaze in early infancy would predict development of ASD in toddlerhood. The study involved a prospective longitudinal sample of infants at high familial risk for ASD and a comparison group of infants at low risk. The researchers recorded electrophysiological brain responses (event-related potentials; ERPs) while